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Interaction of F-actin with phosphate analogues studied by differential scanning calorimetry

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Abstract

The thermal unfolding of F-actin and the changes induced in it by the binding of phosphate analogues were studied by differential scanning calorimetry. It is shown that the conformation of actin is drastically altered by interaction with beryllium fluoride or aluminium fluoride, while the effects of vanadate and phosphate are negligible. The effect of beryllium fluoride on the F-actin structure, as reflected in a significant increase of the actin thermal stability, is much more pronounced in the presence of Mg^{2+} than in the case of F-actin polymerized by KCl or LiCl in the absence of Mg^{2+}. It is concluded that differential scanning calorimetry is a very convenient method for probing the conformational changes in F-actin caused by the interaction with phosphate analogues.

Key words: F-actin; Beryllium fluoride; Aluminium fluoride; Differential scanning calorimetry

1. Introduction

Actin is widely present in all eukaryotic cells and plays a key role in cell motility and cytoskeletal structure. The active form of the molecule is a polymer (F-actin). The polymerization of monomeric actin (G-actin) to F-actin is accompanied by the hydrolysis of bound ATP followed by the slower release of P_i. It has been shown that P_i release destabilizes the F-actin due to the loosening of actin–actin interactions in the polymer [1]. The use of P_i analogues has facilitated studies on the conformation and properties of the F-actin filament in the F-actin-ADP-P_i state. It has been shown that beryllium and aluminium fluoride complexes, the structural analogues of P_i, bind to F-ADP-actin subunits with high affinity, in a 1:1 stoichiometry and in competition with P_i [2]. The structure of the bound complexes was investigated, and the results indicated that AlF_4^- and either $BeF_2(OH)^- \cdot H_2O$ or $BeF_3^- \cdot H_2O$ are the tightly bound species [3].

It has recently been shown that the binding of beryllium fluoride (BeF_x) to F-actin affects the three-dimensional structure of the filament [4] and induces conformational changes in the actin molecule [5]. In accordance with the atomic structure of the actin molecule [6], these changes were localized mainly in the region of subdomain 2 (residues 33–69) [4,5].

In the present work, we have used differential scanning calorimetry (DSC) to study the effects of phosphate and its analogues (beryllium fluoride, aluminium fluoride) and vanadate on the F-actin thermal denaturation. Previously, this method was successfully used to study actin polymerization [7] and the interaction of actin with membrane lipids [8]. In addition we have recently shown that the DSC method enables the probing of global conformational changes of myosin subfragment 1 caused by formation of stable complexes with ADP and orthovanadate [9] or beryllium fluoride [10].

2. Materials and methods

Actin was prepared from rabbit skeletal muscle acetone powder [11]. G-Actin was stored in a low ionic strength buffer composed of 2 mM Tris-HCl, 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM β-mercaptoethanol, and 0.01% NaN_3, pH 8.0 (G-actin buffer). The concentration of actin was determined spectrophotometrically by using $E^{1\%}_{290\ nm} = 6.3$. Generally, the actin samples (2 mg/ml) were polymerized in G-actin buffer by the addition of 4 mM $MgCl_2$ in the presence or absence of 100 mM KCl. In some cases actin was polymerized in the absence of $MgCl_2$ by the addition of 100 mM LiCl [12] or 100 mM KCl. Prior to experiments, F-actin solutions were diluted to a final concentration of 1 mg/ml by the addition of 30 mM HEPES, pH 7.3; ADP was added to a final concentration of 0.2 mM. In order to obtain the complexes of F-actin with beryllium fluoride and aluminium fluoride, F-ADP-actin (1 mg/ml) was incubated for 5 min at 20°C with 5 mM NaF; after addition of $BeCl_2$ or $AlCl_3$ the reaction mixture was further incubated overnight.

Calorimetric measurements were carried out in a differential adiabatic scanning microcalorimeter DASM-4 (Biopribor, Puschino, Russia) as described earlier [9]. All measurements were carried out in 15 mM HEPES, pH 7.3, containing 0.2 mM ADP, at a protein concentration of 1 mg/ml and a constant heating rate 1°C/min. Baselines, obtained by filling both cells with the buffer, were subtracted from the sample experimental traces, thus giving the heat sorption curves. Calorimetric enthalpy, ΔH, was calculated from the area under the heat sorption curve.

3. Results and discussion

F-Actin polymerized with $MgCl_2$ was used in the first set of experiments. Fig. 1 shows the calorimetric data on

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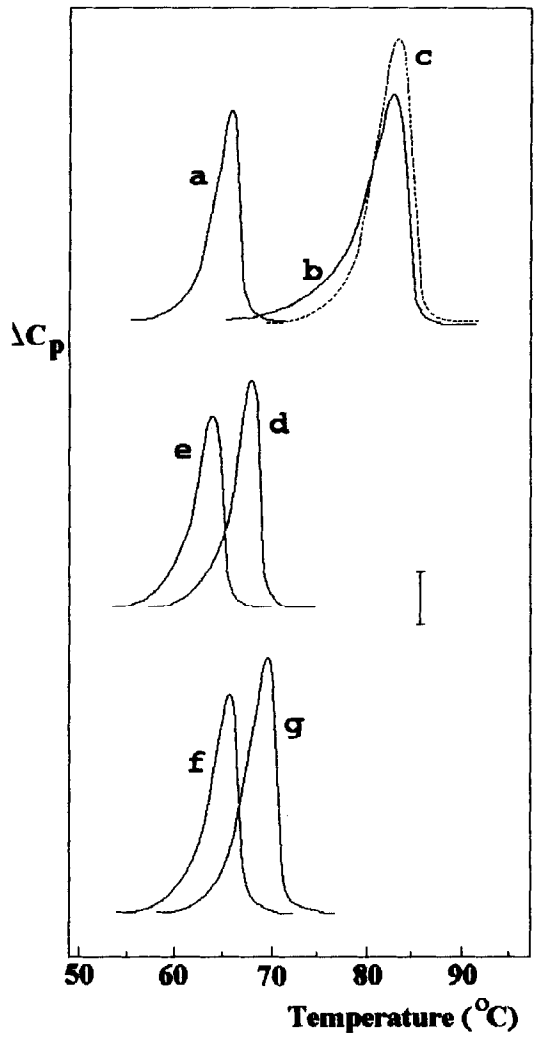

Fig. 1. Temperature dependence of excess heat capacity (ΔC_p) for Mg-F-actin in the absence (a) and the presence of (b) 5 mM NaF, 0.2 mM $BeCl_2$, (c) 5 mM NaF, 0.2 mM $AlCl_3$, (d) 1 mM V_i, (e) 5 mM V_i, (f) 2 mM P_i, and (g) 5 mM P_i. The actin concentration was 1 mg/ml. Conditions: 15 mM HEPES, pH 7.3, 2 mM $MgCl_2$, 0.2 mM ADP, twice-diluted G-actin buffer. Heating rate 1°C/min. The vertical bar corresponds to 50 kJ/K · mol.

the thermally induced denaturation of F-actin containing tightly trapped ADP, and of the complexes of F-actin with phosphate and phosphate analogues. F-Actin alone demonstrates a single sharp transition with a maximum at 65.2°C (Fig. 1a). This transition was irreversible. Our data correlate well with earlier published results [7,8]. In the absence of Be^{2+} or Al^{3+} the addition of NaF caused only a slight increase of F-actin thermal stability, by about 1–3°C (data not shown). On the other hand, the formation of the complex of F-actin with beryllium fluoride in the presence of NaF and $BeCl_2$ results in a change of actin conformation which is reflected in the significant shift of the thermal transition: the maximum of the transition shifts to 82.1°C (Fig. 1b). A similar effect is observed for the formation of the complex of F-actin with aluminium fluoride: in this case the maximum of the transition shifts to 83.1°C (Fig. 1c).

Orthovanadate (V_i), at a concentration of 1 mM, causes a slight increase in F-actin thermal stability, shifting the maximum of the transition up to 67.8°C (Fig. 1d). However, the increase in vanadate concentration led to a decrease in the F-actin thermal stability: in the presence of 5 mM V_i the maximum of the transition shifted to 63.7°C (Fig. 1e). On the other hand, an increase in phosphate concentration from 2 to 5 mM caused an increase in the transition temperature from 65.5 to 69.5°C (Fig. 1f,g). These results are in good agreement with the data of Combeau and Carlier [2] demosntrating that orthovanadate binds to F-actin with the same affinity as phosphate and, at low saturation levels, stabilizes the filament structure in a P_i-like fashion, but destabilizes the filament at higher concentrations. On the other hand, beryllium and aluminium fluorides bind to F-actin with an affinity 3 orders of magnitude higher than phosphate or vanadate [2].

The binding of phosphate and vanadate to F-actin did not significantly change the calorimetric enthalpy of the transition (ΔH = 730 ± 60 kJ/mol). On the other hand, the formation of the complexes of F-actin with BeF_x and AlF_4^- was accompanied by a significant increase in the enthalphy, a ΔH of 950 ± 80 kJ/mol and 1030 ± 80 kJ/mol, respectively. Thus, the complexes of F-actin with BeF_x and AlF_4^- are similar and differ radically from all the other preparations studied. The formation of these complexes results in a significant change of actin conformation which is reflected in a major shift of the thermal transition and in the increase in the enthalphy of the transition.

Beryllium and aluminium fluorides are known to bind to F-ADP-actin subunits with very high affinity, in a 1:1 stoichiometry [2]. The data presented above were obtained in the presence of saturating amounts of BeF_x and AlF_4^- (Fig. 1b,c). Fig. 2 shows results of an experiment in which the concentrations of AlF_4^- are insufficient for complete saturation of all subunits in the F-actin filament. Aluminium fluoride has been chosen to avoid a possible confusion which might arise from using beryllium fluoride, which can exist as different tightly bound species [3,13]. It is shown in Fig. 2 that upon increasing the $AlCl_3$ concentration from 1 to 48 μM, F-actin is increasingly stabilized, as demonstrated by a large increase in the thermal stability. A shift of the thermal transition was observed even at very low $AlCl_3$ concentrations (as little as 1 μM) when less than 5% of F-actin subunits were saturated with AlF_4^- (Fig. 2b). When AlF_4^- was bound to 10–25% of F-actin subunits the transition became much less pronounced (Fig. 2c,d). This wide transition evidently includes many intermediate transitions corresponding to different states of the filament; one of them, with a maximum at about 77°C, is clearly seen in Fig. 2d. However, when about 50% of

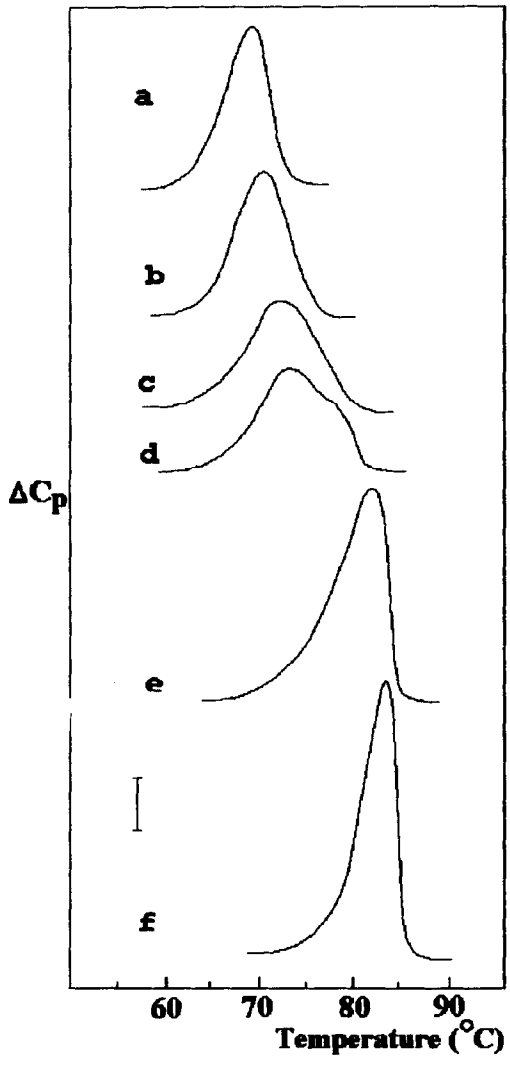

Fig. 2. Temperature dependence of excess heat capacity for Mg-F-actin (24 μM) incubated overnight in the presence of (a) 5 mM NaF and (b–f) 5 mM NaF and increasing concentrations of $AlCl_3$: b, 1 μM; c, 3 μM; d, 6 μM; e, 12 μM; f, 48 μM. Other conditions were as in Fig. 1. The vertical bar corresponds to 50 kJ/K · mol.

F-actin subunits contained bound AlF_4^- the transition became more pronounced (Fig. 2e) and it did not significantly differ from that for F-actin saturated with AlF_4^- (Fig. 2f).

It is important to note the absence of transitions characteristic for control F-actin and for F-actin fully saturated with AlF_4^- in Fig. 2c,d. Recently, we have shown that when the molecules of myosin subfragment 1 (S1) were not fully saturated with V_i, two heat sorption peaks were found, one of which was characteristic of vanadate-free S1 and the other of the S1-ADP-V_i complex [9]. The effect of AlF_4^- is quite different in the case of F-actin (Fig. 2); this suggests the cooperativity of the effect. It seems very likely that the binding of AlF_4^- to some actin subunits not only alters the conformation of these subunits but also induces conformational changes in many neighbouring subunits in the filament. The intermediate transitions at non-saturating concentrations of AlF_4^- can be explained by different denaturation of some parts of the polymer depending on the number of F-actin subunits containing bound AlF_4^-.

Beryllium fluoride binding was shown to affect mainly the conformation of subdomain 2 of the actin subunit [4,5]. On the other hand, the conformation of this smallest subdomain is claimed to depend on the manner of actin polymerization [4,14] and on the divalent cation, Ca^{2+} or Mg^{2+}, present in G-actin [15]. Moreover, some differences were revealed in the effect of BeF_x (protection from tryptic and subtilisin digestions) between Mg-F-actin polymerized in the presence of Mg^{2+} and Ca-F-actin polymerized in the absence of Mg^{2+} (E. Reisler and A. Muhlrad, personal communication). It is reasonable to assume that the effect of BeF_x on F-actin thermal stability should be different depending on the conditions of actin polymerization. In order to check this assumption we have compared the effects of BeF_x on F-actin preparations obtained in different ways.

Fig. 3 shows data on the effect of BeF_x on the thermally induced unfolding of F-actin polymerized in the absence of Mg^{2+}. The thermal transition for Ca-F-actin polymerized with KCl (Fig. 3a) does not significantly differ form that for Mg-F-actin polymerized with Mg^{2+} (Fig. 1a). However, the effect of BeF_x is much less pronounced in this case: the maximum of the transition shifts to 72.7°C (Fig. 3b) instead of 82.1°C characteristic for the complex of Mg-F-actin with BeF_x (Fig. 1b).

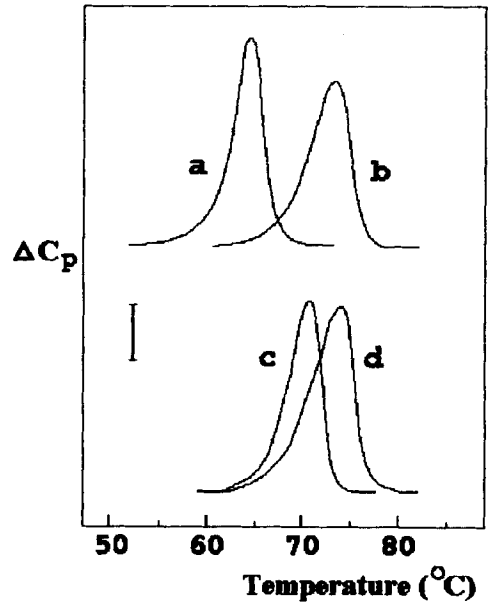

Fig. 3. Temperature dependence of excess heat capacity for F-actin (24 μM) polymerized in the absence of added Mg^{2+} by KCl (a,b) or by LiCl (c,d) and incubated overnight in the absence (a,c) or the presence of 5 mM NaF and 0.2 mM $BeCl_2$ (b,d). Conditions: 15 mM HEPES, pH 7.3, 50 mM KCl or LiCl, 0.2 mM ADP, twice-diluted G-actin buffer. Heating rate 1°C/min. The vertical bar corresponds to 50 kJ/K · mol.

F-Actin obtained by polymerization with LiCl shows the transition with a maximum at 70.3°C (Fig. 3c); BeF_x causes only a slight shift of this peak, up to 73.4°C (Fig. 3d), i.e. to the temperature similar to those characteristic for the transition of the complex of BeF_x with F-actin polymerized by KCl (Fig. 3b). The reason for the minor BeF_x effect in this case is the increased thermal stability of F-actin polymerized by LiCl (Fig. 3c). This observation is consistent with the data of Orlova and Egelman [4] that lithium induces a structural state of subdomain 2 almost indistinguishable from that induced by BeF_x. Comparing these data, one can conclude that the increase in F-actin thermal stability is caused by structural changes in subdomain 2.

It should be noted that the effect of BeF_x on the F-actin thermal stability was different depending on the presence or the absence of Mg^{2+}. A possible decrease in BeF_x affinity for F-actin in the absence of Mg^{2+} is unlikely to be the only reason for this significant difference. We have used an eightfold molar excess of BeF_x over actin, which is usually enough for full saturation of all F-actin subunits under similar conditions [3,4]. Apparently, the effect of BeF_x on the conformation of subdomain 2 strongly depends on the metal bound to actin. The most pronounced effect, reflected in a significant increase of F-actin thermal stability, is observed when Mg^{2+} is the bound metal.

In conclusion, our present calorimetric studies are in good agreement with the results obtained by other methods [2,4,5]. Moreover, it should be noted that the method of differential scanning calorimetry is very convenient for the probing of conformational changes of F-actin caused by formation of its complexes with phosphate analogues.

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